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<b>Protocol Section:</b>	<b>Embryo Cryopreservation</b>	<b>Policy No:</b>	P-CMMR1-ECA-01
<b>Protocol Subject:</b>	<b>Modified Rapid Embryo Freezing Protocol K for ENU and Non-ENU Mice Embryos at Two-Cell Stage</b>	<b>Effective Date:</b>	
		<b>Date Reviewed:</b>	24 October 2004
		<b>Date Revised:</b>	17 May 2006

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This protocol was developed by the Monash University group [L.L.Kuleshova and J.M. Shaw (2000) Human Repr, 15, 2604-2609]. Modifications have been made.

**Media Preparation**

1. Equilibration solution

Prepare a 25% solution of ethylene glycol (EG) [Fisher BP230-1] in PBS [Gibco 21600-069] with 3mg/ml BSA [Sigma A4378]

2. Cryoprotectant (CPA)

Prepare an EG and Ficoll [Sigma F2878] solution in PBS with BSA.  
3.5g of Ficoll is added to 2.5ml EG and 4ml PBS containing 80mg BSA.

3. Thawing media

Prepare a 0.5M sucrose [Sigma S7903] solution in PBS with BSA.

**FREEZING**

1. Collect 2-cell embryos in a minimal volume of PBS.
2. Transfer embryos into the equilibration solution for 2 min at room temperature.
3. Transfer the embryos into the straw pre-loaded with CPA.
4. Heat seal the end of the straw.
5. Plunge the straw directly into LN2.

**Issued by Lab Manager:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Approved by Facility Management:** \_\_\_\_\_ **Date:** \_\_\_\_\_